Vol. XXVII. *November, 1914.* *No. 5*

BIOLOGICAL BULLETIN

IS THE FERTILIZATION MEMBRANE OF ARBACIA EGGS A PRECIPITATION MEMBRANE?

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In 1912 McClendon ('12) suggested that the fertilization membrane of sea urchin eggs is a precipitation membrane formed when two colloids of opposite electrical charge meet, namely the negative egg jelly (mucous, zona pellucida) and a positive substance secreted by the egg. Elder ('13) has advanced a similar view and recently McClendon ('14) has restated his former opinion. Of course the test of the theory is perfectly simple. An unfertilized egg from which the jelly has been removed should form no membrane when fertilized, and both McClendon and Elder state that this is the case.

I have utterly failed to confirm these statements and find that whether an egg forms a normal membrane or not is absolutely independent of the jelly which surrounds it. My method of determining this is as follows:—Eggs from one female are divided into two parts and the jelly removed from the eggs of one part by shaking two or three times in a test-tube and washing with sea water. Eggs of both lots are placed together upon a slide and india ink suspension, sperm, and, after mixing, a cover glass is added. It is perfectly easy to see which eggs are surrounded by jelly and which are not. All of them, whether with or without jelly, form fertilization membranes which in the two-cell stage surround the whole egg and are quite distinct from the hyaline plasma layer which is close to each blastomere. An egg without jelly touching the jelly of another egg should form a membrane, according to McClendon's idea, only on the side toward the jelly. Yet such a condition is never observed, but instead a membrane forms about the whole egg. Thus eggs without jelly will form fertilization membranes.

If *Arbacia* eggs are allowed to stand for about 52 hours at a temperature of 22° C. or for 3 to 4 days at a temperature of 12° C. no membranes form despite the fact that they may still be surrounded by a copious amount of jelly. This observation so far as I am aware was first made by Loeb ('03) and confirmed by myself ('10) and F. R. Lillie ('14). In the two-cell stage the blastomeres are widely separated because not surrounded by a fertilization membrane although the hyaline plasma layer is clearly visible.[1] Thus even an egg surrounded by jelly may fail to form a fertilization membrane.

I at first thought that McClendon's observations were due to the fact that he took so long a time in removing the jelly, (agitation and washing for "not more than 24 hrs.") that the mere age of the eggs would account for their inability to form membranes. I find, however, that the eggs *with jelly* must stand for over 52 hrs. at 22° C. before they are unable to form a fertilization membrane.

This fact suggests that the membrane-forming substance (membranogen) which passes out of the egg to form the membrane gradually diffuses away or is used up when the egg is allowed to stand. Since the membranogen is probably a protein we should expect it to diffuse away from the eggs without jelly much more readily than from those eggs with jelly. It is well known that colloids do not readily diffuse through each other. Such is actually the case and in this point lies, I believe, the explanation of McClendon's results. If we take the eggs from one female, remove the jelly from one lot by shaking, but allow it to remain on the other lot, both lots will form perfectly normal membranes if fertilized immediately. If both lots are allowed to stand for 24 hrs. and are then fertilized the eggs which have stood without jelly form no membranes while those with jelly form membranes only slightly thinner than normal. Membrane

[1] The hyaline plasma layer appears much thicker than in freshly fertilized eggs and in my previous paper ('10) I described this as a special type of membrane. However there is nothing present at all comparable to the normal fertilization membrane and without quibbling over minute distinctions we may safely class these eggs as "without membranes." McClendon ('14) misquotes me in stating that I believe the fertilization membranes to be present on unfertilized eggs and to be simply lifted off after fertilization. I have held exactly the opposite view (Harvey, '10).

formation is not, then, a question of presence or absence of jelly but is dependent on the time the eggs have stood. We may imagine this to be due to the loss of some membrane-forming material from the eggs which much more readily takes place when the mechanical hindrance of the jelly is removed.[1]

These experiments point against the view that the fertilization membrane is a precipitation membrane.

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[1] According to Kite ('12) membrane formation is merely the swelling of a fine invisible (unless stained) vitelline membrane together with a change of its optical properties. If that is true the inability of eggs which have stood for some time without jelly to form fertilization membranes would seem to be due to a loss, through solution, of the vitelline membrane.

SO-CALLED PARTHENOGENESIS IN THE WHITE MOUSE.

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In atretic follicles of ovaries in a number of different mammals the oöcytes go through a process which somewhat resembles maturation. Various stages of mitosis are seen and frequently a first polar body is present. In some cases the oöcyte is found divided up in a number of small parts some of which contain nuclei. This process has been described as a beginning of parthenogenetic cleavage and also as degenerative fragmentation. Bonnet ('00) gives a review of the work done up to that time and after considering all the evidence decides that the mitotic figures seen in such egg-cells are not those of parthenogenetic cleavage but are rather those of more or less abnormal maturation stages. Newman ('13) reviews briefly the work done since Bonnet's paper and presents the results of his studies on the armadillo in support of the view that "a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions." Van der Stricht ('01), who worked on the bat claims that in that form is found a beginning of true parthenogenesis. Rubaschkin ('07), who studied the guinea-pig, and Athias ('09), for the dormouse, state that the phenomena are to be interpreted as degenerative fragmentation which at the most merely resembles parthenogenesis.

In material which I have been preparing for a study of oögenesis in the white mouse I found that in the ovaries of young mice approaching sexual maturity there is a very extensive degeneration of follicles, most marked between the ages of twenty-five and forty days. As the work on this problem has all apparently been done on the ovaries of fully or young adult mammals, it was thought worth while to use the material in these immature ovaries[1] for a study of this so-called parthenogenesis.

[1] The material used for this work consists of ovaries of white mice varying in age from twenty to ninety days. These ovaries were fixed in Carnoy's fixer

In the white mouse, it is certain that the changes taking place in the oöcytes are in some way correlated with the atresia of the follicles, for in follicles which have not begun to degenerate the egg-cells are normal in appearance and the nuclei are normal resting nuclei. But in follicles overtaken by atresia the cytoplasm of the oöcytes is found to stain more deeply with acid stains, fat granules are found in large numbers, and the nuclei are in various stages of mitosis. The degeneration of the follicle in some way stimulates the oöcyte so that it passes through more or less abnormal maturation stages.

The early prophase is probably passed through very rapidly as no stages were seen of nuclei between the resting stage and the equatorial plate stage. Lams and Doorme ('08) did not observe the stages between the resting nucleus and the first polar spindle in the normal maturation of the egg of the white mouse. Kirk-

("6:3:1"), Hermann's, and Flemming's fluids. Heidenhain's iron hematoxylin, Jenner's blood stain, and Flemming's triple stain were used. Sections were stained over night in Jenner's stain, diluted with three parts of water; this stain was used after fixation in Carnoy's fluid and gave excellent results for some purposes. The cytoplasm of degenerating egg-cells was stained a much deeper pink or red than that of normal egg-cells. The nuclei of follicle cells and phagocytic cells were stained a deep blue. The stain will fade after a time, however.

A modification of theshorter method of Flemming's triplestain was used. Sections of material fixed for two to four hours in Flemming's or Hermann's fluid were bleached in a dilute solution of hydrogen peroxid and after rinsing were placed in a four per cent. solution of ferric alum for four to twelve hours. The sections were then rinsed in distilled water, dipped in the safranin solution a second or two, rinsed again in distilled water, and placed in the gentian violet solution for two to ten or fifteen minutes. Then after rinsing in distilled water the sections were stained in the orange G for ten to sixty seconds. After dehydrating rapidly in absolute alcohol the sections were differentiated in clove oil under the control of the microscope. The clove oil was removed by toluene or xylene and the sections mounted in balsam. By this method the cytoplasm of the oöcytes was stained a yellow-brown, the chromosomes were stained a violet, and the spindle fibers a dark violet—almost purple. The method is rather capricious but when successful, the spindle fibers stand out very distinctly against the yellow-brown cytoplasm of the egg-cell. The method was used principally to bring out the spindle fibers, as the chromosomes are not stained so distinctly or sharply as by the iron hematoxylin method.

The solutions used are as follows:

1. { Safranin, saturated solution in absolute alcohol, 1 part.
   { Safranin, saturated solution in distilled water, 1 part.
2. Gentian violet, 1 per cent. solution in distilled water.
3. Orange G, 2 per cent. solution in distilled water.

ham ('08) describes very briefly a few stages of the prophase of the first maturation division in this same form.

Descriptions of the first and second polar spindles in normally maturing oöcytes do not agree. Sobotta ('07) says: "Wenn auch namentlich die Breite individuell etwas wechselt, so beträgt Länge wie Breite des ersten Richtungsspindel der Maus doch stets das Doppelte der Masse des zweiten Richtungsspindel, die Breite ist in der Regel mehr als doppelt so gross." Lams and Doorme, on the other hand, find that the first and second polar spindles are of exactly the same length and diameter. They state: "D'une façon certaine, un ovule à second fuseau ne se distingue d'un ovule à premier fuseau que par la présence, dans le premier cas, du premier globule polaire." Kirkham agrees with Sobotta in saying that the first polar spindle is larger than the second and also in describing the chromosomes as differing in size and shape in the two spindles.

In regard to oöcytes in atretic follicles, Athias says of the spindle, first or second: "Sa forme et ses dimensions sont variables, mais il n'y a pas des caractères qui soient propre au premier ou au second fuseau; la présence concomitante d'un premier globule polaire est, d'après ce que j'ai pu constater dans mes préparations, le seul critérium pour affirmer si l'on est en présence d'un second fuseau de direction." In my own preparations the lengths and diameters of a number of first and second polar spindles in atretic oöcytes were measured. For the first polar spindles, the average length was found to be 24.7 μ, the diameter 12.8 μ, while for the second, the average length was 25.9 μ and the diameter 8.7 μ. Allowing for error in measuring, the spindles are seen to be of about the same length, while the second polar spindles are about two thirds the diameter of the first.

In degenerating oöcytes of the white mouse the first polar spindles are found to be of two general forms: slender, with the achromatic fibers meeting at a point at each end, and thick, "barrel-shaped," with broadly rounded ends. These two kinds are met with in about equal numbers. In both, the chromosomes are arranged with their long axes parallel to that of the spindle. The chromosomes are not arranged around the periphery of the

spindle, but are scattered in the plane through the middle of the spindle, at right angles to its length. The chromosomes of the first polar spindle are larger than those of the second and are long with a marked thickening or swelling at the middle. This thickening is at one side of the chromosomes and it is at this point that division takes place. The division is apparently transverse. This account agrees with those of Sobotta and Kirkham for the normal first maturation spindle.

The chromsomes are grouped so closely together and overlap to such an extent that it is difficult to determine their number with accuracy. They apparently vary in number from twelve to twenty-four, the larger numbers being due to a precocious division of some, while others are still undivided.

Descriptions of the appearance of the spindle itself differ. Sobotta, Kirkham, and Lams and Doorme, state that polar radiations or asters are not present in first polar spindles of normally maturing oöcytes, and Athias agrees with them in the case of degenerating egg-cells. Rubaschkin, however, describes in atretic oöcytes of the guinea-pig polar radiations arising from a clear area or centrosphere. Kirkham describes centrosomes consisting of several minute granules at the poles of the spindles. Sobotta states that there are no centrosomes in the first polar spindles of normally maturing oöcytes, and Athias finds none in degenerating egg-cells. In my own preparations radiating fibers are to be seen at the poles of a few first polar spindles; these, however, are not to be considered true asters, but spindle fibers which have broken away from the spindle. This will be discussed more in detail further on. No centrosomes are found in any of these degenerating oöcytes. The spindle fibers are not divided into central spindle fibers and mantle fibers; the fibers with chromosomes attached are intermingled with those which are not connected with chromosomes. The two kinds do not differ in appearance or staining reaction. The spindle itself usually lies at right angles to the radius of the oöcyte, until it swings around to a radial position for the formation of the polar body. (See Figs. 1, 2 and 5.)

The stages in the formation of the first polar body must follow one another rapidly for only a few of these stages were

observed. Some of the chromosomes divide earlier than others and consequently the metaphase is not so distinctly marked as in some forms. A few instances of a telophase were seen, in some of which both groups of daughter-chromosomes are in the oöcyte, with no indication as yet of a division of the cytoplasm to form the polar body, while in the others the constricting off of the polar body may be plainly seen. After the first polar body is formed the chromosomes remaining in the oöcyte do not form a resting nucleus but at once enter the second polar spindle.

The second polar spindle, as stated above, is of about the same length as the first, while only two thirds or three fourths as much in diameter. The chromosomes as seen in the equatorial plate stage are short and rod-like and straight or slightly curved. They are not as long or as much curved as Kirkham describes in the second polar spindles of normally maturing oöcytes. The chromosomes are arranged with their long axes parallel to that of the spindle itself and as in the first polar spindle are scattered in a plane at right angles to the length of the spindle. This differs from Kirkham's account of the normal second polar spindle, in which he states that in general the chromosomes lie with their long axes across the spindle. In the spindles of these degenerating egg-cells some of the chromosomes are constricted across the middle in preparation for division, having the appearance of elongated dumb-bells; others have not started to divide and have the typical rod-like form. Others still have already divided and are short and thick, their length only slightly exceeding their diameter. (See Figs. 3 and 4.) This division as well as the first is apparently transverse in the mouse. The chromosomes are crowded together, as in the first polar spindle, making an accurate count difficult; there are from twelve to thirty, owing to the fact that some divide sooner than others.

Descriptions of the appearance of the second polar spindle are as conflicting as those of the first. Sobotta states that in normally maturing oöcytes there are neither centrosomes nor polar radiations. Lams and Doorme describe centrosomes but say that polar radiations are absent; Kirkham states that both centrosomes and polar radiations are present in some cases. In second polar spindles of degenerating oöcytes Athias states

that neither centrosomes nor polar radiations are present. In my own preparations radiating fibers are to be seen at the poles of a number of second polar spindles. As in the case of the first polar spindles, however, these are not to be considered true asters, but spindle fibers which have broken away from the spindle, and have assumed a radial position at the ends of the spindles. This will be discussed more in detail later. In general, centrosomes are absent in second polar spindles of atretic oöcytes, but are present in some cases. When they are seen, they consist of a few minute granules which stain deeply and are either in a compact group at the poles of the spindle or are somewhat spread out forming a sort of cap for the spindle. (See Figs. 3, 4 and 6.)

The first polar body is almost always present with the secondary oöcyte in atretic follicles, although in a few instances the spindle has all the characteristics of a second polar spindle while the polar body is not to be seen. In such cases it is possible that the polar body has already degenerated and been absorbed, or as Kirkham suggests for normal oöcytes, it may have been "forced through the zona (pellucida) by the contraction of the latter under the influence of changing osmotic conditions" during fixation. In nearly every instance, however, the polar body is present, lying within the zona pellucida, and somewhat flattened between the oöcyte and the zona. The smaller dimension of the polar body is one half or two thirds of the larger, while the larger diameter itself is a fifth to a third that of the oöcyte. In a few cases the polar body contains a spindle more or less deranged and abnormal (see Fig. 3) but usually the chromosomes are scattered through it irregularly. They may be grouped in a few large irregular masses of chromatin or there may be a number of smaller chromosomes of abnormal size and shape. In a few cases a resting nucleus may be seen in the polar body (Fig. 10). The second polar spindle is usually found in the oöcyte near the polar body, at right angles to the radius of the egg-cell. Rarely it may be seen in the other side of the oöcyte, and a few spindles have been seen in a radial position. A few instances were observed where the oöcyte contained two spindles; this is probably due to the fact that the egg-cell had two nuclei to start with.

Up to this point oöcytes in follicles undergoing follicular atresia have passed through the same stages, with some differences in detail, as normally maturing egg-cells. The later stages however are different. The next step in degeneration is the breaking down of the spindles. The usual course is for the oöcytes to form the second polar spindles which then break down; but if degeneration has proceeded a little more rapidly, this fate may overtake the first polar spindles before the polar body is formed.

As stated above, in the first polar spindle as well as in the second, the achromatic fibers are all intermingled, those with chromosomes attached and those without, and do not differ in appearance or staining reaction. The fibers with no chromosomes attached to them break across at their middle and the free ends move out in the cytoplasm. As the polar ends remain attached to the poles of the spindles, the formation of "asters" is brought about. Stages are seen (Figs. 5 and 6) in which the breaking or splitting off of the fibers is taking place; some of the fibers have just broken across, in the figures, and others have already assumed a radial position, giving the appearance of "asters." The achromatic fibers with chromosomes attached next break or split off, and as their free ends move out into the cytoplasm, they draw after them the attached chromosomes (see Fig. 7). In this way more fibers are added to the "asters" and chromosomes are seen connected with the ends of some of the fibers. This splitting off of the achromatic fibers explains the fact that some spindles have radiating fibers or "asters" at their poles, while in others they are absent. The oöcytes containing spindles without radiating fibers have not advanced so far in degeneration that the spindle fibers have begun to split off. The result of this splitting off of the fibers and the consequent breaking down of the spindles is that the chromosomes are scattered in all directions in the cytoplasm at each pole of the spindle, while still connected with the poles by the spindle fibers.

The cause of the breaking down of the spindles is to be found in the degeneration of the oöcyte. That this degeneration has proceeded to quite an extent is shown by the presence in the cytoplasm of fat-granules and crystalloid bodies, and by the

fact that the cytoplasm stains much more deeply with acid stains such as eosin and orange G than does the cytoplasm of normal oöcytes. The spindle fibers share in this degeneration and show it first by breaking across and splitting off from the spindle. Rubaschkin states that as the fibers split off, the poles of the spindles approach each other and finally come to lie so close together that it is difficult or impossible to distinguish one from the other. While this account of the breaking down of the spindles agrees essentially with that of Rubaschkin for the guinea-pig, nothing resembling the approach of the poles of the spindles was observed in the mouse.

The achromatic fibers soon disappear and the chromosomes thus left free in the cytoplasm of the oöcyte begin to form nuclei. Each chromosome forms a small vesicle which has the appearance of a vacuole with the chromatin material massed at one side (Fig. 13). In some instances the chromatin is arranged in small granules around the outer part of the vesicle (Fig. 8). As this process goes on, the vesicles near enough together coalesce to form larger ones (Figs. 8, 9, 11), while those isolated in the cytoplasm remain separate. In this way a varying number of nuclei are formed, of different sizes. A nucleus formed by the combining of a number of chromosomes is larger than one formed from a single chromosome. The final number of nuclei thus formed may be from two to twelve, depending on how the chromosomes were scattered in the oöcyte. These nuclei are transformed into resting nuclei of more or less normal appearance.

The nucleo-cytoplasmic relationship, already interfered with by the degenerative changes in the egg-cell, is further disturbed by this formation of a number of small nuclei. The size-relationship, as well as the morphological, physiological, and chemical, relationship, is clearly affected. Apparently there is an effort, even in the degenerating oöcyte, to restore as far as possible this size-relationship, and this effort is expressed by a breaking up of the cytoplasm into smaller parts around the various nuclei. A part of the cytoplasm may surround several of these small nuclei when these are close together, or may enclose only one, when they are isolated. It occasionally happens that a bit of the cytoplasm may fail to contain even one of these nuclei, when

part of the egg-cell was without any nuclei as a result of an incomplete scattering of the chromosomes. The result of this breaking up of the oöcyte is that there are formed a number of small "cells," some with several nuclei, some with one, and some with none, so that the oöcyte has the appearance of a "morula." The fact that some "cells" have nuclei and others have not, is due to the uneven distribution of the chromosomes in the cytoplasm of the oöcyte when the spindle breaks down. In general, the "cells" containing large nuclei, or a group of nuclei, are larger than those with one nucleus or none. However, a definite or effective control over this fragmentation is apparently lacking.

Several authors, Newman among others, have described cells in this "morula" stage which have spindles in them, and state that these are cleavage spindles and that therefore this is a case of parthenogenetic cleavage. It is more probable, however that in such cases the cell containing the spindle is the first polar body, which, as noted above, occasionally forms a spindle, and which may in rare instances divide. In the white mouse no spindle was found in any of the cells of this "morula" stage.

In the cells of the "morula," and sometimes in the oöcyte before it has fragmented, are frequently found crystalloid bodies the nature and origin of which are unknown. Possibly they are a product of the degenerative changes in the egg-cell. Fat granules are found in the oöcytes in increasing numbers as degeneration goes on.

In a few instances the oöcyte is found to have formed two cells of nearly equal size, each containing a nucleus. Van der Stricht describes such cases in the bat and states that each cell may divide again, and each of the four cells thus formed may also divide. The formation of two such equal cells may be explained on the grounds that the scattered chromosomes were arranged in two groups and formed two nuclei; the oöcyte then broke up into two fragments of equal size. Such an egg-cell is shown in Fig. 10, with the first polar body also present; but the two nuclei are not equal in size, nor normal in appearance. In fact, one is apparently little more than a vacuole.

The fate of the "morula" may be briefly described. The zona pellucida usually persists as a thick transparent membrane

for some time after the oöcyte itself has completely degenerated and disappeared, although in a few instances it is absorbed early. Phagocytic (?) cells make their way into the oöcyte through the zona pellucida, and are probably to be regarded as follicle cells from the degenerating follicle. These cells are usually seen in the outer border of the oöcyte, or just outside it, lining the inner surface of the zona pellucida, sometimes as early in the course of degeneration as the spindle stage. There are not many of these cells in a single oöcyte, not more than eight or ten and frequently no more than three or four. They may be imbedded in the cytoplasm of the egg-cell and in the "morula" stage are frequently seen in between the separate cells. One case is illustrated (Fig. 14) showing one of these extra-ovular cells just after it has entered the oöcyte, still retaining its connection with other follicle cells outside the egg-cell by means of a protoplasmic process extending through the zona pellucida. This same cell is also shown to be connected with one or two other similar cells within the zona by other protoplasmic processes, forming a sort of syncytial net-work or mesh-work in among the fragments of the degenerating egg-cell.

The cytoplasm of these cells is usually rather scanty and sometimes they look like bare nuclei imbedded in the cytoplasm of the oöcyte (Figs. 8 and 9). They are not, however, to be confused with the nuclei of the oöcyte formed by the breaking down of the spindle, for they react differently to the stains used and have a different structure. They are finely granular and these granules are stained an intense black by iron hematoxylin and deep blue by Jenner's stain.

It may be through the action of these cells that the fragments of the oöcyte are gradually absorbed and disappear, for later on the zona pellucida is seen, shrunken and distorted, with a few of these cells in a remnant of the egg-cell. In still later stages, these cells are seen alone inside the zona, and this condition may persist for some time (Fig. 16). Eventually the zona pellucida and these cells all disappear and by this time the follicle itself has usually completely degenerated.

Thus it is seen that the oöcytes in atretic follicles in the ovary of white mice not yet sexually mature undergo a series of changes

which in the early stages at least resemble maturation. That these changes are in some way correlated with the atresia is shown by the fact that all the oöcytes exhibiting these phenomena are found in atretic follicles, and egg-cells of normal appearance are seen in the follicles not yet overtaken by atresia. The degeneration of the follicle stimulates the oöcyte to pass through a process which at first resembles maturation but which later results in a breaking up of the egg-cell into fragments, some with nuclei and some without. In the light of the evidence here presented, this process can not be considered parthenogenetic cleavage for no mitotic figures other than those of a more or less abnormal maturation were seen; and if this were true parthenogenetic cleavage it would be expected that some stages of mitosis would be observed. The absence of mitotic figures other than more or less normal polar spindles, the breaking down of these spindles, the scattering of the chromosomes, the formation of nuclei from these chromosomes, and the consequent breaking up of the egg-cell into small parts, with or without nuclei, show rather conclusively that in the white mouse, not yet sexually mature, the process is one of degenerative fragmentation.

Summary.

The spindles seen in oöcytes in follicles undergoing atresia folliculi are maturation spindles, more or less abnormal, and not cleavage spindles.

By the splitting off of the achromatic fibers and the consequent breaking down of these spindles the chromosomes are scattered through the cytoplasm of the oöcyte and form a number of nuclei.

The nucleo-cytoplasmic relationship, disturbed by the degenerative changes in follicle and egg-cell, causes the oöcyte to break up into fragments, some with one or more nuclei and some with none. These fragments are gradually absorbed, probably through the action of phagocytic cells of follicular origin, and disappear.

The process is one of degenerative fragmentation and not parthenogenetic cleavage.

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EXPLANATION OF PLATES.

All the figures are camera-lucida drawings made from the actual preparations. All the drawings except Figs. 3, 7, and 14, were made by Miss Cora J. Whitman.

PLATE I.

FIG. 1. Primary öocyte, containing a first polar spindle. The chromosomes are longer and more slender than usual in first polar spindles. The zona pelludica is seen surrounding the egg-cell (zp.). × 670.

FIG. 2. Primary oöcyte containing a first polar spindle, of the "barrel-shaped" type. The chromosomes are of the type usual for this spindle. The zona pellucida has disappeared. × 670.

FIG. 3. Secondary oöcyte, with second polar spindle and polar body which also contains a spindle more or less deranged. Some of the chromosomes of the egg spindle have been omitted from the drawing in order to show more clearly the characteristic shape of the chromosomes of the second polar spindle. × 670.

FIG. 4. Secondary oöcyte, with second polar spindle and polar body. Centrosomes are seen at each end of the spindle. × 916.

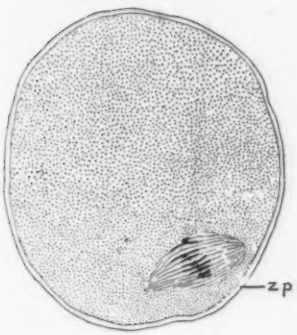


Fig 1

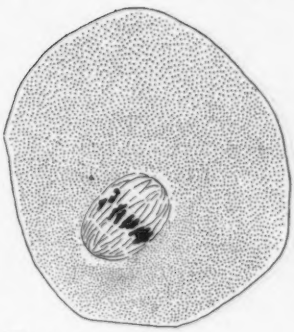


Fig 2

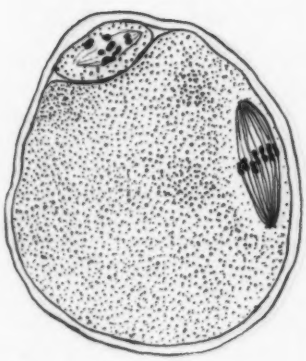


Fig 3

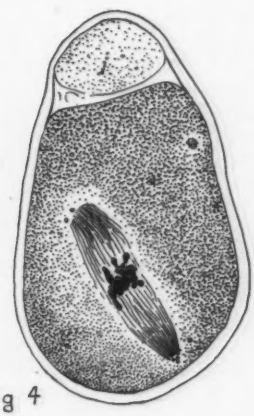


Fig 4

H. M. KINGERY.

PLATE II.

FIG. 5. First polar spindle alone, showing achromatic fibers splitting off, forming "asters." × 916.

FIG. 6. Second polar spindle alone, showing well-defined "asters," formed by fibers which have split off from the spindle. × 916.

FIG. 7. Primary oöcyte showing spindle seen obliquely from one end, which has broken down. The scattering of the chromosomes is partially accomplished. ×670.

FIG. 8. Egg-cell with nuclei formed from scattered chromosomes. The bodies at the end of the oöcyte (*x*) are probably cells formed by the division of the polar body. A crystalloid body (*c*) and an extra-ovular cell (*p*) are also shown. ×670.

Fig. 5

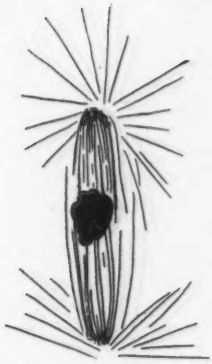


Fig. 6

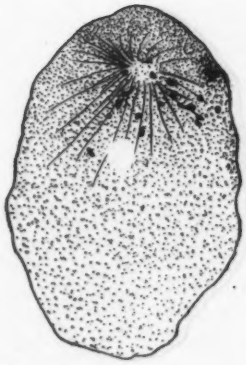


Fig. 7.

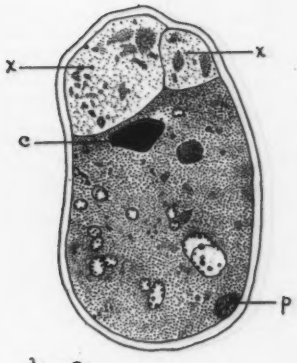


Fig 8.

PLATE III.

FIG. 9. Egg-cell containing four nuclei; these, from their size, have been formed by the coalescence of a number of smaller vesicles. Two extra-ovular cells (*p*) are shown. × 670.

FIG. 10. Oöcyte divided into two more or less equal parts, with polar body also present (*pb*). The nuclei are not alike, one being apparently only a hollow vesicle. × 670.

FIG. 11. Egg-cell in several fragments, one of which contains five nuclei. The zona pellucida is broken in two places and extra-ovular cells are present between the fragments. × 670.

FIG. 12. "Morula" stage, some fragments with nuclei and others without. × 670.

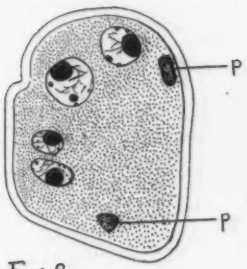


Fig 9

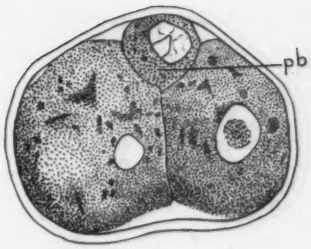


Fig 10

Fig 11

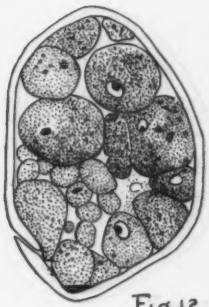


Fig 12

PLATE IV.

FIG. 13. Egg-cell showing small nuclei formed from the scattered chromosomes. The chromatin material is massed at one side of each of the vesicles. × 670.

FIG. 14. Egg-cell containing extra-ovular cells connected by protoplasmic processes. One is still connected with the follicle cells outside by a process extending through the zona pellucida. × 670.

FIGS. 15 AND 16. Final stages in degeneration. Zona pelludica with remnant of oöcyte and a few extra-ovular cells inside. × 670.

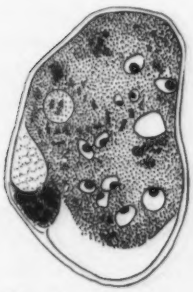


Fig. 13

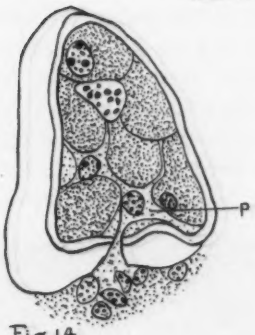


Fig 14

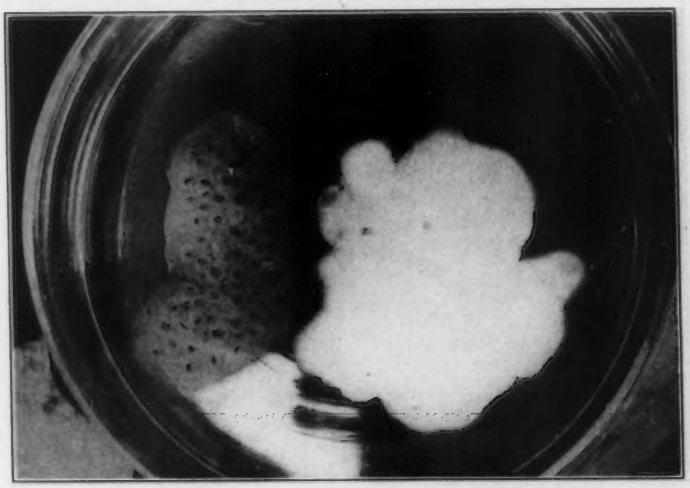
Fig 15

Fig 16

A MILKY WHITE AMPHIBIAN EGG JELL.

ARTHUR M. BANTA AND ROSS AIKEN GORTNER.

While collecting *Ambystoma punctatum* eggs in April, 1914, the writers found a freshly-laid bunch which had jell of milky whiteness instead of being transparent. The white clutch of eggs was conspicuous among the others even though the jell had as yet swelled very little and the bunch was quite small. The eggs were still undivided. They had the normal amount of pigment. The jell was so clouded that at first no eggs could be seen in the mass without breaking into the jell. The jell imbibed water and the egg bunch soon assumed the normal proportions for the



clutch of this species. The photograph shows the relative opacity of the white bunch and a normal bunch of eggs of the same age. Only a few eggs were visible in the opaque bunch when the photograph was taken while one could readily see the bottom of the dish through the jell of the normal bunch. The individual outer and inner egg membranes in the white bunch were of normal transparency. This is indicated indistinctly

in the photograph by the somewhat more transparent spots, in which were eggs, near the edges of the white bunch.

When just ready to hatch the embryos with their egg membranes were removed from the jell, care being taken to remove all traces of eggs which had died during development. The water was carefully drained off and the jell was dried in a porcelain dish over a water bath. The jell from a normal egg bunch of the same stage of development was treated in a similar manner. After drying on the water bath the jell was further desiccated in a vacuum desiccator over sulfuric acid.

233 grams of normal egg jell yielded 0.7855 grams or 0.337 per cent. of dry material.

294 grams of the opaque jell yielded 1.060 grams or 0.361 per cent. of dry material.

It thus appears that the jell which normally surrounds these eggs contains about 99.6 per cent. of water and yet this jell is fairly tough and resistant and admirably suited to supporting the eggs in such a position as to permit ready diffusion of oxygen and carbon dioxide and to protect the eggs from mechanical injury.

The two jells when desiccated were indistinguishable. A portion of each was again placed in water and in a short time they had imbibed enough water to resume their former appearance, the one becoming milky white and the other transparent. It may perhaps be germane to add that the milky appearance could not be ascribed to bacteria inasmuch as the egg mass was found shortly after being deposited and also because there was no evidence of bacterial decomposition up to the time the drying was begun.

Thinking that perhaps the milky color was produced by an admixture of albumen with the mucin (which composes the normal egg jell) nitrogen determinations were made of both abnormal and normal egg jells.

The nitrogen was determined by Kjeldahl's method.

Normal egg jell.—0.2060 gram gave 12.3 c.c. 0.1 normal NH_4OH; 0.3564 gram gave 21.1 c.c. 0.1 normal NH_4OH, indicating 8.36 per cent. and 8.29 per cent. respectively or an average of 8.32 per cent. of nitrogen in the normal egg jell.

Opaque egg jell.—0.2764 gram gave 18.3 c.c. 0.1 normal NH_4OH; 0.3540 gram gave 23.0 c.c. 0.1 normal NH_4OH, indicating 9.27 per cent. and 9.09 per cent. respectively or an average of 9.18 per cent. of nitrogen in the opaque egg jell.

Almost no chemical work has been done with the amphibian egg jells aside from the observation that the nitrogen content is low due to the presence of a carbohydrate nucleus. Of course the above results do not give the true nitrogen content of the egg jell for they should have been corrected for ash content, but this was not possible due to lack of material. The figures do show, however, that the jells differ chemically as well as in appearance, and that the difference in nitrogen content is in the same direction and of almost precisely the same amount that it would be if the opaque appearance were produced by an admixture of albumen (nitrogen = 15 per cent.–16 per cent.) with the normally occurring mucins.

STATION FOR EXPERIMENTAL EVOLUTION,
August 17, 1914.

THE RELATION OF THE BODY TEMPERATURE OF THE EARTHWORM TO THAT OF ITS ENVIRONMENT.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

In a paper by one of us upon the "Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals"[2] the assumption was made that the temperature of the living animal (worm or fish-embryo) under observation corresponded very closely to that of the water surrounding it. The same assumption has been made by other workers in this field, *e. g.*, Snyder[3] in his work upon the isolated heart of the Pacific terrapin assumed that the temperature of the more or less bulky heart muscle of the terrapin was conditioned by the temperature of the solution in which it was placed. Robertson[4] also in his work upon *Ceriodaphnia* assumed that the temperature of the water definitely represented the temperature of the tissues with which he was particularly concerned. Even more recently Loeb and Ewald[5] make use of the same assumption.

There are to be found in the literature of physiology statements concerning the body temperatures of the so-called cold-blooded animals, and an examination of the data offered reveals the fact that the various investigators who have taken the trouble to make any examination of the actual conditions find that the temperatures of the animals studied vary considerably from the temperatures of the surroundings. It is also true that many

[1] From the Department of Zoology, Oberlin College.

[2] Rogers, Charles G., "Studies upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals," *American Journal of Physiology*, 1911, Vol. 28, No. II., pages 81–93.

[3] Snyder, C. D., "On the Influence of Temperature Upon Cardiac Contraction and Its Relation to Influence of Temperature upon Chemical Reaction Velocity," University of California Publications, Physiology, Vol. 2, No. 15, 1905.

[4] Robertson, T. B., "Note on the Influence of Temperature Upon the Rate of the Heart Beat in a Crustacean (*Ceriodaphnia*)," BIOLOGICAL BULLETIN, 1906, Vol. X., No. 5, pp. 242–248.

[5] Loeb, Jacques and Ewald, W. F., "Die Frequenz der Herztätigkeit als eindeutige Funktion der Temperatur," *Biochemische Zeitschrift*, 1913, Bd. 58, H 3, 177–185.

of the observations recorded were made by means of mercurial thermometers, though some were made by thermocouples with galvanometers. In reading the statements of the different investigators one is left with the feeling that with the more improved appliances of the present day one ought to be able to make observations which would be more satisfactory than those published. The following table summarizes the results of a number of investigators, and is compiled from data quoted by Milne-Edwards.[1]

Animal.		Authority.
Fishes	less than 1°C. above surrounding water	Milne-Edwards
Frog	0°.32–2°.44 " " "	Czermak
Frog	0°.0 –0°.575 " " "	Becquerel
Frog	0°.04 " " "	Dutrochet
Toad	0°.2 " " "	"
Frog	0°.7 –0°.3 " " "	Dumeril
Proteus	1°.25 " " "	Rudolphi
Proteus	2°.6 –5°.6 " " "	Czermak
Crayfish	6°.0 " " "	Rudolphi
Maia sq.	0°.3 –0°.9 " " "	Valentin
Limax	0°.33–0°.50 " " "	Spallanzani
Snail	2°.0 " " "	Hunter
Snail	1°.1 " " "	Martine
Snail	0°.9 " " "	Becquerel
Snail	1°.5 –2°.0 " " "	Schnetzler
Aplysia	0°.1 –0°.8 " " "	Valentin
Octopus	0°.2 –0°.6 " " "	"
Eledone	0°.9 " " "	"
Annelids	0°.56–0°.85 " " "	Hunter
Lumbricus	1°.11–1°.39 " " "	"
Holothuria	0°.2 –0°.6 " " "	Valentin
Ophiurian	0°.3 " " "	"
Asterias ru.	0°.6 " " "	"
Sea Urchin	0°.4 –0°.5 " " "	"
Medusa Pelagia	0°.2 –1°.0 " " "	"
Medusa Cassiopea	0°.3 " " "	"
Actinians	0°.2 –0°.5 " " "	"

In the table it will be noted that the temperatures determined for the different animals show, for the most part, rather small variations from that of the surrounding water. In a few cases the variation is quite considerable, and appears to make desirable a reëxamination of the facts. This is especially true in view of

[1] Milne-Edwards, "Leçons sur la Physiologie et L'Anatomie Comparée de L'Homme et des Animaux," T. VIII., Paris, 1863.

the fact that certain scientific friends have raised question as to the validity of the assumption upon which the temperature coefficient work was based. It is with an idea of attempting to answer any questions as to the propriety of assuming that the temperature of the earthworm is represented by the temperature of the surroundings, that the present investigation is here reported.

Methods.

A method of measuring differences of temperature by means of the electromotive force developed when the junctions of wires of different metals of a common circuit are not at the same temperature was described by Nobili and Melloni[1] about 1830. Since that time galvanometers have been made more sensitive, and it has also been made possible to obtain pure metallic wires of small diameter, and of small heat capacity. The authors named were the first to apply this method of temperature measurement to living animals, and now that the methods of use have been somewhat improved the same method has been employed for the measurement of the amount of heat given off in a given contraction of a frog muscle.[2] The method can be made accurate enough to measure differences as small as 1/150° C. For the purpose of the investigation here reported it did not seem necessary to make measurements as small as those recorded by Hill, so the number of junctions of the wires was not at all increased. The thermo-couples used consisted of No. 32 copper and No. 32 constantan wires joined together as shown in Fig. 1. In some of the couples the wires were simply twisted together and in others the junction was made secure by a small drop of solder. We were not able to determine that for our purpose the soldered junctions were any more efficient than those not soldered. The junction used within the body of the worm was mounted within a slender glass tube in such a way as to have the two wires of the couple thoroughly insulated from each other except at the junction point (Fig. 2). This was accomplished by placing

[1] Nobili et Melloni, "Recherches sur plusiers phenomenes calorifiques enterprises au moyen du thermo-multiplcaiteur," *Ann. de chimie et de physique*, 1831, T. XLVIII., p. 208.

[2] Hill, A. V., "The Energy Degraded in the Recovery Process of Stimulated Muscles," *Journal of Physiology*, 1913, Vol. 46, pp. 28–80.

one of the wires in a finely drawn glass tube, allowing the end of the wire to extend slightly below the end of the tube, where it was twisted together with the other wire of the couple. The small glass tube with the two wires was then placed inside another glass tube of a diameter just sufficient to receive them easily. The glass of the outer tube was then sealed over the junction of the wires, and the whole bent into a convenient form for handling. The upper open ends of the tubes were sealed with wax to prevent the access of any water and the apparatus was ready for use.

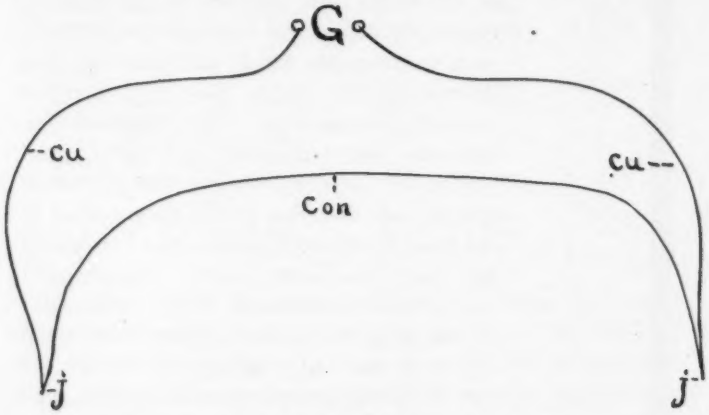


FIG. 1. Diagrammatic scheme of thermocouple. *G* = galvanometer; *cu* = copper wire; *con* = constantan wire; *j* = junction.

The galvanometer used in this work was one of the D'Arsonval type made by Gaertner. It was provided with a dead beat coil, and was so heavily damped that it was found desirable to allow two or three minutes for the galvanometer mirror to come to rest when making observations. It was found that with the scale at about 1 meter distance 1° C. difference in the temperature of the two junctions of the thermo-couple was represented by a shifting of the reading of the galvanometer scale by about 16 millimeters. The actual amount of shift varied somewhat from day to day from this value, and was re-determined for each day's work.

The technic of determining the temperature of the interior of

the worm was very simple. It consisted in placing the glass-covered junction of the thermocouple in the mouth of the worm, and carefully pushing it down through the œsophagus, crop and gizzard until it came to lie in the stomach intestine. The other junction of the couple was placed in water of a known temperature, and the two end wires of the thermocouple were attached to the galvanometer. The temperature of the water in which the free junction of the thermocouple was placed was determined by a Beckman thermometer which had been set to a definite reading by the side of a certified standard thermometer. The temperature of the water was determined to 1/100° C., and was noted for every observation so that in case of any variation in the temperature of the water we should be able to make the necessary correction in the results. The temperature of the worm was shown by the amount of the deviation of the galvanometer reading, from the zero reading, divided by the number of millimeters representing 1° C., and adding this amount to the known temperature of the water. In this way we were able to make readings which could be accepted as accurate to within 0.03° C., which for our purpose seemed to be sufficient.

FIG. 2. Sketch of thermo-electric clinical thermometer for use with the earth-worm. *cu* = copper wire; *con* = constantan wire; *j* = junction.

The following table gives data derived from three experiments to show how closely and how rapidly the temperature of the worm becomes adjusted to the temperature of the water in which it is immersed. We have not thought it necessary to multiply examples as all the facts observed are in perfect harmony with those offered. In the experiments here cited both the worm and the free junction of the couple were placed in the same bath. The difference between the zero and in-circuit readings of the

galvanometer would then indicate the difference in temperature between the temperature of the water and the interior temperature of the worm if any such difference exists. After each reading the worm and free junction of the couple were placed in the water of the temperature indicated for the next following reading.

Time.	Temp. of Water in Which Worms Were Placed.	Zero Reading of Galvanometer.	Galvanometer Reading Couple in Circuit.	Difference in Millimeters.	Temperature of Worm.
A. 9.40	11.40° C.	345	345	000	11.40° C.
10.10	21.30	345	345	000	21.30
B. 9.00	21.24	403			
9.17	21.24	403	402	001	21.30
9.25	10.00	403	403	000	10.00
9.35	21.20				
9.40	21.20	408	408	000	21.20
9.55	13.00	408			
10.00	13.00	408	408	000	13.00
C. 10.30	21.20	403	404	001	21.14
10.40	17.00	403	404	001	17.94
10.45	21.20	403	404	001	21.14

The above data are representative of a large number of observations made during an investigation upon the effect of temperature changes upon the rate of contraction of the dorsal blood vessel of the earthworm, *Lumbricus agricola*, and indicate very clearly that the animal under investigation adapts itself with remarkable quickness and closeness to the temperature of its environment. In fact we think it may safely be said that the worm will adapt itself to a change of at least ten degrees Centigrade within two minutes, to an accuracy of 0.05° C. This fact makes it possible then to use the temperature of the water surrounding the animal as an indicator of the temperature of the animal, in the case of the earthworm, for experiments upon the temperature coefficient of heart action, and assures us that the worm need not be subjected to a bath of a given temperature for any great length of time in order to get an accurate result. It is very likely true that the same principle will be found to hold good for other animals of a similar kind and habit, certainly for marine worms, fish embryos, small crustacea, etc. It is the purpose of the authors to continue the investigation upon other

forms in order to determine to what extent we may be at liberty to assume the temperature of the surrounding fluid to be an indicator of the temperature of the tissues.

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THE TEMPERATURE COEFFICIENT OF THE RATE OF CONTRACTION OF THE DORSAL BLOOD-VESSEL OF THE EARTHWORM.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

Certain unpublished criticisms of a previous paper of the senior author[2] have led us to make a further investigation of the effects of changes of temperature upon the rate of contraction of the dorsal blood vessel of the earthworm. The criticism offered against the previous work was that no evidence was presented to show that the temperature of the forms studied, worms and fish-embryos, was the same as that of the water in which they were immersed. The force of the criticism is recognized, and we are now able to present the results of an investigation in which the temperature of the worm studied was determined by means of a delicate clinical thermometer inserted in the long, tubular, alimentary canal of the worm.

We are now publishing under another title[3] an account of the work in which it is shown that the temperature of the surrounding water does furnish an excellent indicator of the inner body temperature of the earthworm, when this animal is immersed in water for experimental purposes. Inasmuch as this is true we have no doubt that the previous work upon *Nereis*, *Tubifex*, and the embryos of *Fundulus* and the toad-fish will bear the same sort of inspection.

We will not at this time take up any discussion of the literature of the subject. The papers of Snyder, Robertson, Loeb and Ewald and others are available for examination. The formula employed for the computation of the temperature coefficients

[1] From the Department of Zoology, Oberlin College.

[2] Rogers, Charles G., "Studies Upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals," *American Journal of Physiology*, 1911, Vol. XXVII., pp. 81–93.

[3] Rogers, Charles G., and Lewis, Elsie M., "The Relation of the Body Temperature of the Earthworm to that of its Surroundings," BIOLOGICAL BULLETIN, 1914, Vol. XXVII., pp. 261–267.

is the one used by Snyder in all his work, and concerning which he has presented some matters of historical interest,[1]

$$Q_0 = \left(\frac{K_1}{K_0}\right)^{\frac{10}{T_1 - T_0}}.$$

The material used in this study was the large earthworm, *Lumbricus agricola*. This form presents two desirable features for this work; 1st, the animals are not by nature restricted to any definite or narrow limits of temperature at which their normal physiological processes take place; and 2d, it is well adapted structurally for the work in that it is easily possible to place a delicate temperature measuring apparatus in the alimentary canal, and it is also easy to see the contractions of the dorsal blood vessel through the more or less transparent body wall. This last fact is of special importance as it makes it practicable to leave the animal undisturbed in its constant temperature bath, thus obviating any disturbance of the vascular contractions through nervous action due to stimulation from without.

Methods.

As a preliminary to the actual temperature work a number of worms were subjected to immersion, for varying periods, in water to ascertain what harmful effects might result. Without going into detail as to this work it may be stated that the worms are able to withstand immersion in tap water for a sufficient time to allow all the experimentation needed for the temperature studies, without showing any harmful effects. In fact certain worms have been immersed for as long a period as two weeks without showing any injurious effects.

The temperatures of the worms were regulated, then, by placing them in baths of water, the temperature of which was controlled by placing the dishes in thermostats having practically constant temperatures, in refrigerators cooled by ice, or in the running water of the laboratory, which was found to have a very constant temperature.

The temperatures of the worms were determined by means of

[1] Snyder, C. D., "On an Interpolation Formula Used in Calculating Temperature Coefficients for Velocity of Vital Activities, Together with a Note on the Velocity of Nerve Conduction in Man," Science, N.S., Vol. XXXIV., No. 874, p. 415.

delicate clinical thermometers, in the form of thermo-couples, which could be inserted into the mouths of the worms and pushed on down into the stomach intestine. These thermo-couples were made of No. 32 copper and No. 32 constantan wires. Any difference in the temperature of the two junctions of the couple sets up an electromotive-force proportional in its strength to the amount of the temperature difference, and which can be accurately measured by means of a delicate galvanometer. In this particular work it was found that 1° C. was represented by a shift in the reading of the galvanometer scale of about 16 mm. As it was practicable to read to half millimeters it will be seen that a temperature difference of 0.03° could be determined. For a somewhat more detailed account of the method of temperature measurements the reader is referred to another paper.[1]

The worm having been subjected to a given temperature for a sufficient length of time to have become completely adjusted to the new condition, the temperature of the worm was noted, and the length of time required for a definite number of beats of the dorsal blood-vessel, usually 25, was taken by means of a stop-watch reading to fifths of a second. The worm was then changed to another bath at a different temperature, allowed to remain long enough to become thoroughly adjusted to the new condition, and another reading of the rate of contraction of the dorsal blood vessel made. From the data thus obtained the temperature coefficient of the rate of contraction was calculated by means of the formula referred to above.

It may be said that all possible precautions were taken to avoid serious errors in the work. Temperatures were determined by making the readings of the galvanometer against a certified thermometer calibrated to tenths of a degree C. The temperatures of the various baths were kept as uniformly constant as possible and the exact temperature taken each time a count was made. Occasionally one is in doubt as to whether a contraction of the dorsal blood vessel has actually taken place. In such a case the reading was thrown out and another made so as to make sure of the fact. The temperature of the room was kept as constant as possible in order to avoid any changes of resistance

[1] Rogers, Charles G., and Lewis, Elsie M., *l. c.*

in the copper wires and in the galvanometer which might tend to disturb the results. Manipulation of the worms was reduced to a minimum in order that nervous effects might not be introduced to invalidate the temperature effects. Reading of the rates of contraction of the dorsal blood vessel before and after the insertion of the glass covered junction indicated that the mere insertion of the instrument in the alimentary canal made no difference with the rate.

TABLE I.

No. of Worm.	K_1	K_0	T_1	T_0	Q_{10}
3	19.35	13.74	21.43°	11.60°	1.406
5	15.00	9.80	18.00°	11.50°	1.925
6	20.35	10.90	18.00°	11.50°	2.593
7	21.10	13.80	16.04°	11.50°	2.548
8	15.72	8.92	16.04°	11.50°	2.096
9	22.00	9.93	16.04°	11.50°	5.766
10	16.93	10.60	16.04°	11.50°	2.805
11	17.75	11.29	16.04°	11.50°	2.642
12	14.79	11.76	16.04°	11.50°	1.656
14	27.00	19.40	26.40°	11.70°	1.272
16	26.90	14.92	26.40°	11.60°	1.485
17	15.27	11.13	26.50°	11.60°	1.236
18	19.70	15.33	26.50°	11.60°	1.176
19	29.30	12.50	27.13°	12.55°	1.793
21	24.15	13.50	27.12°	12.55°	1.280
24	33.60	24.00	27.00°	16.36°	1.372
26	37.80	26.70	26.60°	24.60°	5.754
27	32.56	19.35	20.00°	9.50°	2.044
28	35.27	24.55	20.00°	9.50°	1.413
29	21.39	8.85	27.50°	14.00°	1.621
30	28.20	12.13	27.10°	14.00°	1.899
31	20.40	13.18	27.70°	14.00°	1.365
32	27.48	11.75	27.30°	14.04°	1.898
33	21.50	12.66	26.50°	14.02°	1.525
34	26.55	14.05	25.60°	14.03°	1.733
35	22.00	12.95	25.08°	13.88°	1.605
36	24.86	12.63	25.08°	13.92°	1.838
37	16.97	8.95	22.54°	13.95°	2.109
38	20.40	11.58	22.46°	13.96°	1.947
39	22.43	11.86	21.86°	13.94°	2.236
40	21.59	9.81	21.43°	13.95°	2.871
41	26.80	11.16	22.90°	13.72°	2.603
42	21.97	11.10	22.95°	13.74°	2.099
43	23.33	11.94	22.71°	13.80°	2.121
44	25.00	11.23	23.18°	13.73°	2.388
Average value of temperature coefficient for all specimens.....					2.173

The preceding table, Table I., gives the data derived from the actual experiments and also the values of the temperature coefficient calculated from the data. In the tables the letters

K_1 and K_0 indicate the rates in contractions per minute at the temperatures T_1 and T_0 respectively, in degrees Centigrade.

If we arrange the data of Table I. so as to show the relation existing between the higher and lower temperature ranges and the value of Q_{10} we have evidence that for the lower temperatures there is a higher coefficient than for the higher ranges. This fact is not so clearly shown in the case of the earthworm as in some of the forms already studied, probably for the reason that it is very difficult to count the beats of the dorsal blood vessel of the earthworm at temperatures below 8° C. The movements of the walls are so feeble and so slow that one is not sure when a contraction has taken place.

TABLE II.

T_1.	T_0.	Number of Worms.	Average Q_{10}.
About 27° to about	14°	4	1.671
" 27° " "	12°	2	1.536
" 26° " "	11°	4	1.292
" 25° " "	13°	3	1.725
" 22° " "	13°	6	2.199
" 21° " "	13°	2	2.533
" 20° " "	9°	2	1.728
" 16° " "	11°	6	2.919

In Table II. we have such an arrangement of the data as suggested in the preceding paragraph. It will be noted that while in general the coefficients for the lower ranges are higher than those for the higher ranges of temperature, there are very marked exceptions to the rule. The only explanation we have to offer at this time for the rather marked variation from what we should expect, if the beat of the dorsal blood vessel of the earthworm is subject to the same laws as the beats of various hearts already studied, is that we have here to deal with a series of nervous effects which must be in some way eliminated in order to avoid complications. Up to the present time we have not found any means of avoiding these nervous effects in the worm, though in fish embryos where the rate of heart beat was studied before the nervous connections were established it was found that one could predict with some degree of certainty what the rate of heart contraction should be at any stated temperature.

That the rate of beat of the dorsal blood vessel of the earthworm is to some extent under the control of the nervous system will be shown in another publication from this laboratory.

It is to be noted also that the temperature coefficient of the rate of contraction of the dorsal blood vessel of the earthworm is, for the temperatures at which the worm would naturally live, of the same general magnitude as those of chemical reactions, and the average for the whole series, 2.173, also falls within the limits usually set for the temperature coefficients of such reactions. We have no reason, as yet, to assign for the much reduced coefficients for the higher temperature ranges.

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AUDITORY POWERS OF THE CATOCALA MOTHS; AN EXPERIMENTAL FIELD STUDY.[1]

C. H. TURNER AND E. SCHWARZ.

Historical Resumé.

Near the close of the nineteenth century, Romanes ('91) wrote: "Among insects organs of hearing certainly occur, at least in some, although the experiments of Sir John Lubbock appear to show that ants are deaf. The evidence that some insects are able to hear is not only morphological, but also physiological, because it is only on the supposition that they do that the fact of stridulation and other sexual sounds being made by certain insects can be explained, and Brunelli found that when he separated a female grasshopper from the male by a distance of several meters, the male began to stridulate in order to inform her of his position, upon which the female approached him. I have myself published observations proving the occurrence of a sense of hearing among the Lepidoptera."

The tone of three fourths of the above paragraph is characteristic of practically all of the early works upon the auditory powers of insects. Those men were convinced that insects hear; not because they had experimentally demonstrated it, but for morphological reasons, and because many kinds of insects can produce sounds. They believed that an insect would not be endowed with the power of producing sounds unless the other members of the species could hear. At first in the Orthoptera and later in other groups of insects, peculiar organs were found; consisting essentially of vibratory hairs attached to certain cells that seem to be sensory in nature. In some cases these hairs are in cavities and in others they are not. Such was the nature of the work of Siebold ('44), Leydig ('55), Henson ('66), Lee ('83, '85), Graber ('75, '82), Weinland ('91), Adelung ('92) and others. As late as 1905 Radl expressed the following thought. No matter

[1] For the identification of the species and for the experimental work on *C. unijuga*, E. Schwarz alone is responsible; the field work was performed jointly; for the planning of the work, for the historical resume and for the method of treatment, C. H. Turner is solely responsible.

how often in recent years doubt has arisen as to the ability of insects to hear, it has been largely maintained that they possess an auditory sense, and for the following two reasons: (1) the ability of many insects to produce sounds as a part of their normal behavior; (2) the possession by insects of organs which structurally seem fitted to act as receptors of sound waves.

Students interested in the morphological method of investigating this question will find the paper by Radl ('05) intensely interesting. After epitomizing the work by Lee ('83, '85), by Graber ('82), and by Weinland ('91), he states that, on a priori grounds, he doubted the assumptions of Graber; but that certain experiments had convinced him that insects have a crude auditory sense. In support of his contention, he offers the following reasons.

1. Graber is inaccurate when he claims that the chordotonal organs are located rigidly between two immovable parts of the body; for the proximal end is attached to an indifferent part of the body, while the distal end is in close proximity to one or more muscles.

2. The chordotonal organ cannot function like a string attuned to a certain pitch; because it expands and contracts.

3. All of the chordotonal organs examined are attached at each end to a chitinous framework and the nerve penetrates from the side.

4. The chordotonal organs resemble somewhat those muscles which occur especially in the limbs of the Arthropoda—muscles which terminate in long tendons.

5. No chordotonal organ is found in either the Myriapoda or the Arachnida.

6. Chordotonal organs are found in some insects for which a sense of hearing could have no significance. They are well developed in caterpillars; even in those of the Tortricidæ, which spend the entire larvel period inside of some fruit. They are also well developed in the internal parasites of certain insects.

7. All attempts to determine experimentally that insects react to pure and simple tones have yielded negative results: however, it is comparatively easy to evoke responses of insects to shrill noises, such as the voice of a cricket or the screech produced by

drawing a file across the edge of an iron or a glass plate. This is not a tactile reaction.

8. There is no evidence that noise, as such, causes the orientation of insects. The sounds produced by insects are more an outburst of inner feeling than an attempt to entice the female by the male.

9. The outcome of the whole matter is that there is an auditory sense in insects; but, it is on a much lower plane of development than that of the vertebrates. Its anatomical and physiological antecedents are to be found, not in the tactile organs and contact activities; but, anatomically in sense organs which register muscle activities and physiologically in general sensation (Gemeingefuhlen). The auditory sense of insects is a highly refined muscular sense.

Although the work of the early investigators was largely, often entirely, morphological, it must not be concluded that no experimental work has been done on the auditory powers of insects and their near kin. Such experiments have been performed in several groups of insects; but the results are inharmonious. Buttel-Reepen ('00) and De Fraviere believe that bees can hear. Buttel-Reepen's statement is based upon his observation that bees respond in a definite manner to the sounds of their own kind.

Huber ('10) and Forel ('03) interpret their experiments to mean that ants cannot hear. Lubbock's experiments likewise yielded negative results; yet, in spite of this, he was unwilling to admit that ants, wasps and bees cannot hear. Weld ('99) thinks he has experimentally demonstrated that ants can hear. Fielde and Parker ('04) interpret their experiments to mean that ants do not respond to sound vibrations as such. C. H. Turner ('07) is equally positive that his experiments demonstrate that ants can hear. At one time Wheeler believed, not only that ants can hear; but that they communicate by means of sounds; but, after the appearance of the paper by Fielde and Parker, he ('10) asserts that there is not sufficient evidence to warrant the assumption that ants can hear.

E. A. Andrews ('11) is convinced that termites hear. He writes: "In a community suspended from the ceiling by a copper

wire and represented by many thousands on a moist block of artificial stone which they got to from the nest by means of a long stick as bridge, it was first observed by Mr. Middleton that the noise of thunder and of blasting rocks was followed by a quick and very remarkable departure of almost all of the termites towards the nest. The blocks of stone weighed some sixteen pounds each and rested in a large pan of water on a firm wall of stone, so that it seemed likely that the concussion of the air came to the termites directly and not as a tremor of the stones they were clustered on. The same precipitous flight of the multitude of termites from these stones to the nest along the bridge was brought about by dropping a board upon the concrete floor with a loud crash. Even the clapping of hands, which probably shook the stone foundation but imperceptibly, served to drive the termites back to the nest. . . . Attempts to influence the termites by blowing horns of various pitches near them failed though considerable disturbance of the air was produced."

Montgomery ('10), after reviewing all that had been written on the auditory powers of spiders, concludes that spiders are deaf.

The above is not an exhaustive discussion of the published research work on the auditory powers of insects other than the Lepidoptera; yet, we trust it is sufficient to show the inharmoniousness of the results of different investigators.

To the best of our knowledge, the first published results of experiments upon the auditory powers of butterflies and moths is an article by Romanes ('76) which appeared about thirty-six years ago. The portion referring to the Lepidoptera is so short that we quote it in full. "It seems worth while to add a few words with respect to the sense of hearing in insects. So far as I am aware, the occurrence of such a sense in this class has never been actually proved. Although on *a priori* grounds there can scarcely be any doubt concerning the fact of some insects being able to hear; seeing that in so many species stridulation and other sounds are made during the season of courtship. In the case of moths, however, I believe that sounds are never emitted—except, of course, the death's head moth.[1] It therefore becomes inter-

[1] Romanes was mistaken when he asserted that the death's head moth is the only Lepidopteran than produces sounds; for the literature contains records of

esting to observe that an auditory sense is certainly present in these insects. Several kinds of moths have the habit of gently, though very rapidly, vibrating their wings, while they themselves are at rest on a flower or other surface. If, while this vibrating movement of the wings is going on, the observer makes a sudden shrill note with a violin, or fife, etc., the vibrating movement immediately ceases, and sometimes the whole body of the insect gives a sudden start. These marked indications of hearing I found invariably to follow a note with a high pitch, but not a note with a low one."

Heinrich ('09) remarks that collectors using a net to capture *Limenitis populi* and *Catocala fraxini* have observed that the insects often take flight before a collector is near enough to capture them. Accordingly to him no conclusive evidence has been published on the sense of hearing of insects, especially of the Lepidoptera. He noticed a *Laurentia suffumata* alight in a concert pavilion and remain quietly while the brass band played three selections, one of which was Wagner's Götterdammerung. He also noticed that certain Lepidoptera were more easily approached at twilight than when the sun was shining brightly, and he could not understand why this should be true if they were warned by a sense of hearing. He is convinced that, in all of these cases, it is vision, not audition, that warns butterflies and moths of the approach of man.

Hamann ('09) was led to investigate this subject by the remarks of collectors that butterflies and moths undoubtedly hear. One collector remarked that the noise caused by removing the cork from the cyanide bottle often caused these insects to fly. To this Haman replies that since the net is usually placed beneath the insect before the cork is removed it is probable that the sight of the net caused the flight. To test the matter, the following experiments were performed by him. (1) He approached a tree in such a manner as to be invisible to an *Apatura iris* L. resting thereon, and struck the tree with the bamboo handle of his

several species of Lepidoptera that emit sounds. Indeed, scarcely had his article appeared before several of his contemporaries published, in *Nature*, protests in which were cited several examples of sound-producing Lepidoptera. Recently Omensetter ('12) and Stephan ('12) have described several sound-producing forms of butterflies and moths.

collecting net. To this the insect made no response; but, as soon as the net approached it took flight. (2) a repetition of the experiment with *Vanessa* yielded similar results. (3) He noticed that certain Catocalas, which were not disturbed by the noise of a passing automobile, flew upon the approach of man. These experiments convinced him that butterflies and moths cannot hear.

Deegener's work ('09) is morphological. Between the thorax and the abdomen, on the ventral side of the body of all species of Nocturidæ, there is a depression containing chitinous structures and hairs which are connected with what seem to be sensory cells. A careful examination of this organ in *Pseudophia lunaris* convinced Deegan that it is probably an auditory organ.

Rothke ('09) confined a *Limenitis artemis* in a cage which he placed on the top of a pedestal two feet high. The sides and back of this cage were constructed of wood; but the front was covered with wire fly netting. At nine P.M., while the front of the cage was illuminated by means of a kerosene lamp, Rothke stepped to one side and made a slight noise. To this the insect made no response. The investigator then tapped rapidly and sharply upon the floor with a leather slipper. Although the jar was not sufficient to shake the stand upon which the cage rested, and although the investigator could not be seen by the insect, yet it slowly raised its wings until they met above its back and then lowered them again. Several repetitions of this experiment yielded identical results. During the intervals between the experiments the creature remained immobile. After the moth had been quiet for one quarter of an hour, Rothke rapped upon the table with a tumbler. Immediately the insect flapped its wings. About midday he discovered a specimen of *Catocala unijuga* resting quietly, about six feet from the ground, upon a pine tree one and a half feet in diameter. He picked up a stone about fifteen centimeters in diameter and threw it against the tree-trunk. Although the moth could not see the stone and although the blow was too slight to jar such a large tree, yet the moth flew away. Rothke is convinced that butterflies and moths can hear.

Observations made upon *Catocala pacta* L. convinced Richter

('09) that the crackling of twigs under his feet and even the slight noise made by removing the cork from his collecting bottle disturbed this moth. In another article, the same investigator ('10) has made a comparative study of the auditory powers of day-flying and night-flying Lepidoptera. He investigated the day-flyers; *Apatura* sp., *Vanessa* sp., *Limenitis populi* L., and *Sat. alcyone* Schiff. He found that species of *Apatura* and of *Vanessa* made no responses to sound so long as no visible object disturbed them. During a severe storm, he noticed a number of *Sat. alcyone* perching on a limb. Neither whistling, nor the clapping of hands, nor shaking of the limb had any effect on them: but, as soon as the hand of the collector approached them, they flew. A *Vanessa antiopa*, resting on a telegraph pole, was not disturbed by the shrill whistle and the rumbling noise of a passing train. In studying *Catocala fraxini* L., a night-flying moth, he noticed that it made no responses to the noises made by wagons, automobiles and the bells of a ferry; but that it responded readily to slight, high-pitched, sounds. He argues that the failure of this moth to respond to the sounds made by wagons and such things is because such sounds have no life significance for the moth. On the other hand, the ready response to the other sounds mentioned is due to their similarity to sounds made by field-mice, bats and owls—sounds which for the moth have a pronounced life significance. Partly influenced by the knowledge that Geegener had discovered chordotonal organs in the Noctuidæ and more so by the observations just described, Richter is convinced that day-flying Lepidoptera are warned by visual and night-flying forms by auditory stimuli.

For years Rober ('10) has raised *Acerontia atropos* L. from pupae. Late one evening a female emerged and, before her wings were fully dried, a male emerged in the cage. In order to separate the two, the female was removed to a cage in the bottom of which there was a crack as wide as one's finger. These cages were three meters apart. In the morning the female, which had escaped from confinement, was found perched on the cage containing the male. A person who slept in the room with these two moths asserts that, for a long time that evening, those moths emitted sounds. Rober concludes that these were love calls and that the

moths mutually heard. In face of the well known fact that the sense of smell is well developed in butterflies and moths, the evidence just cited does not appear to be conclusive.

Is it possible for anyone to read the above historical resume and not be convinced that there is need for much exact experimentation upon the auditory powers of insects? Evidently the last word has not been spoken.

Description and Discussion of Experiments.

Reading Stephan's ('12) recent articles on sound producing butterflies and moths induced in us the same thought that influenced the opinions of many of the early investigators; namely, animals that produce sounds as a part of their normal behavior can probably hear. We decided to make some crucial experiments. The Catocala moths were selected for the following reasons: (1) one of us is so well acquainted with the taxonomy of the group that it is easy to identify species afield; (2) the habit these moths have of resting during the day on some tree trunk and, when disturbed, flying to a nearby tree trunk renders them ideal material for field experiments.

Preliminary Observations.

Observations afield taught us that there are certain sounds to which these moths do not respond. A favorite haunt of the Catocala moths of this vicinity is a small stretch of wood through which a railroad passes. Moths resting on trees near the tract are not disturbed by the whistle, rumble and roar of passing trains. Near that same place there is a pleasure garden in which the sounds of a noisy piano are often heard. No responses to the strains of the piano were noticed.

These observations are in harmony with those of Heinrich ('09), Hamann ('09) and Richter ('09, '10).

Indoor Experiments on Catocala unijuga.

Three specimens freshly hatched from pupae were the subjects of these experiments. Each was kept in a separate room. Three times a day, for four days, the auditory powers of these moths was tested by whistling in a high key. Occasionally the moth was shielded from the draft of air caused by whistling;

TABLE I.

Catocala unijuga. SPECIMEN NUMBER 1.

Number of the Experiment.	Date.	Whistled, the Insect Shielded from the Air Currents.	Whistled, the Insect Not Shielded from the Air Currents.	Number of Times it Was Necessary to Whistle Before Response Was Received.
1	June 5, 1913, 10:00 A.M....	*N*		
2	" " " ...		*F*	1
3	" " " ...		*F*	1
4	" " " ...	*F*		4
5	" " " ...	*Q*		5
6	June 5, 1913, 10:30 A.M....		*F*	2
7	" " " ...	*Q*		6
8	" " " ...	*F*		3
9	" " " ...	*Q*		8
10	June 5, 1913, 6 P.M.....	*F*		4
11	" " "	*Q*		3
12	" " "	*F*		6
13	" " "	*Q*		10
14	June 6, 1913, 9:00 A.M.....	*N*		
15	" " "	*Q*		4
16	" " "	*F*		2
17	" " "	*Q*		5
18	June 6, 1913, 11.00 A.M....	*F*		4
19	" " " ...	*Q*		10
20	" " " ...	*F*		4
21	" " " ...	*Q*		15
22	June 6, 1913, 6:00 P.M.....	*F*		8
23	" " "	*Q*		15
24	" " "	*F*		6
25	" " "	*Q*		19
26	June 7, 1913, 9:00 A.M.....	*F*		4
27	" " "	*Q*		10
28	" " "	*F*		4
29	" " "	*Q*		6
30	June 7, 1913, 10:00 A.M....	*F*		3
31	" " " ...	*Q*		8
32	" " " ...	*F*		3
33	" " " ...	*Q*		15
34	June 7, 1913, 7:00 P.M.....	*F*		6
35	" " "	*Q*		5
36	" " "	*F*		4
37	" " "	*Q*		6
38	June 8, 1913, 10:00 A.M....	*F*		2
39	" " " ...	*Q*		10
40	" " " ...	N_4		
41	" " " ...	*Q*		6
42	June 8, 1913, 10:30 A.M....	*N*		
43	" " " ...	*Q*		6
44	" " " ...	N_2		
45	" " " ...	*Q*		10
46	" " " ...	*F*		2
47	June 8, 1913, 6:00 P.M.....	*F*		4
48	" " "	*Q*		3
49	" " "	*F*		6
50	" " "	*Q*		10
51	" " "	*F*		13

EXPLANATION OF TABLES I–III.

N indicates no response; *F* means that the moth flew; *Q* indicates that the insect waved its wings up and down or made some quivering movement.

TABLE II.

Catocala unijuga. SPECIMEN 2.

Number of the Experiment.	Date.	Whistled, the Insect Shielded from the Air Currents.	Whistled, the Insect Not Shielded from the Air Currents.	Number of Times it Was Necessary to Whistle Before the Moth Responded.
1	June 6, 1913, 9:00 A.M.....	*Q*		6
2	" " "		*F*	2
3	" " "	*F*		5
4	" " "	*Q*		4
5	June 6, 1913, 10:00 A.M....		*F*	1
6	" " " ...	*F*		4
7	" " " ...	*Q*		3
8	" " " ...	*F*		2
9	June 6, 1913, 6:00 P.M.....	*F*		1
10	" " "	*Q*		3
11	" " "	*F*		2
12	" " "	*Q*		6
13	June 7, 1913, 10:00 A.M....	*F*		2
14	" " " ...		*F*	
15	" " " ...	*F*		4
16	" " " ...	*Q*		5
17	June 7, 1913, 11:00 A.M....	*F*		2
18	" " " ...	*QF*		4
19	" " " ...	*F*		4
20	" " " ...	*Q*		4
21	June 7, 1913, 6:00 P.M.....	*F*		3
22	" " "	*Q*		3
23	" " "	*F*		2
24	" " "	*Q*		8
25	June 8, 1913, 9:00 A.M.....	*N*		
26	" " "		*F*	1
27	" " "	*Q*		3
28	" " "	*F*		8
29	June 8, 1913, 11:00 A.M....	*F*		4
30	" " " ...	*Q*		6
31	" " " ...	*F*		4
32	June 8, 1913, 7:00 P.M.....	*F*		10
33	" " "	*Q*		6
34	" " "	*F*		2
35	" " "	*Q*		6
36	June 9, 1913, 9:00 A.M.....		*F*	2
37	" " "	*Q*		10
38	" " "	*F*		2
39	" " "	*Q*		10
40	June 9, 1913, 11:00 A.M....		*F*	2
41	" " " ...	*Q*		9
42	" " " ...	*F*		1
43	" " " ...	*Q*		11
44	June 9, 1913, 7:00 P.M.....	*Q*		6
45	" " "	*F*		1
46	" " "	*Q*		6
47	" " "	*F*		10

TABLE III.

Catocala unijuga. SPECIMEN NUMBER 3.

Number of the Experiment.	Date.	Whistled, the Insect Shielded from the Air Currents.	Whistled, the Insects Not Shielded from the Air Currents.	Number of Times it Was Necessary to Whistle Before the Response.
1	June 7, 1913, 9:00 A.M.....	*N*		
2	" " "		*F*	1
3	" " "	*Q*		3
4	" " "	*F*		2
5	" " "	*Q*		10
6	June 7, 1913, 10:00 A.M....	*F*		2
7	" " " ...	*Q*		6
8	" " " ...	*F*		2
9	" " " ...	*Q*		10
10	June 7, 1913, 7:00 P.M.....	*F*		2
11	" " "	*Q*		10
12	" " "	*F*		4
13	" " "	*Q*		15
14	June 8, 1913, 9:00 A.M.....	*F*		3
15	" " "	*Q*		6
16	" " "	*F*		4
17	" " "	*Q*		10
18	June 8, 1913, 10:00 A.M....	*N*		
19	" " " ...	*Q*		10
20	" " " ...	*F*		6
21	" " " ...	*Q*		5
22	June 8, 1913, 10:00 P.M....	*F*		6
23	" " " ...	*Q*		4
24	" " " ...	*F*		4
25	" " " ...	*Q*		10
26	June 9, 1913, 9:00 A.M.....	*F*		4
27	" " "	*Q*		3
28	" " "	*F*		6
29	" " "	*Q*		10
30	June 9, 1913, 11:00 A.M....	*F*		6
31	" " " ...	*Q*		10
32	" " " ...	*F*		5
33	" " " ...	*Q*		8
34	June 9, 1913, 6:00 P.M.....	*F*		10
35	" " "	*Q*		2
36	" " "	*F*		4
37	" " "	*Q*		6
38	June 10, 1913, 9:00 A.M....	*Q*		1
39	" " " ...	*N*		
40	" " " ...	*Q*		6
41	" " " ...	*F*		2
42	" " " ...	*Q*		7
43	June 10, 1913, 11:00 A.M...	*N*		
44	" " " ...	*Q*		3
45	" " " ...	*F*		1
46	" " " ...	*Q*		11
47	June 10, 1913, 6:00 P.M....	*N*		
48	" " " ...	*F*		2
49	" " " ...	*Q*		6
50	" " " ...	*F*		1
51	" " " ...	*Q*		10

but, in some cases, the air current was allowed to strike the moth. The results of those experiments are recorded in tables I.–III.

On the twelfth of June the three specimens, which, up to that time, had been confined in separate rooms, were marked and placed in the same room. At nine A.M. that day, on the first sound of the whistle, they all flew, one after another, as though the flight of the first had evoked the flight of the others. At ten o'clock, the whistling caused two to fly and the other to quiver. The one that quivered was about ten feet away. On whistling again all flew. At six P.M. the whistling caused all to fly.

To our way of thinking this series of experiments is very instructive. That each of these three specimens responded to the whistle on the twelfth of June is unequivocal; that they usually responded to the whistle by either flying or by quivering is also evident; but, it is equally certain that two out of the three specimens did not respond to the whistle at all the first time it was sounded and that the third specimen responded in a feeble manner. When the moths did not respond to the blowing of the whistle at the beginning of the experiment, the current of air produced by whistling was allowed to strike the moth; immediately it flew, and thereafter it would usually fly when the whistle was sounded. There were some exceptions to this; but, in the main it was true. This seems a hint that the moth responds to sounds that have a life significance.

Field Experiments.

These experiments were conducted in a small stretch of woods at Meramec Highlands, near St. Louis, Mo. Previous experience had taught us that these insects would not respond to loud sounds of low pitch. For that reason we used as the sound producing instrument a Galton whistle set to give a high shrill note. One of us would stand where the moth could be observed; but far enough away not to disturb it. Experience had taught us what would be a safe distance. The other, whistle in hand, would approach the tree on the opposite side to that on which the moth was resting. When this experimenter was near to the tree the whistle was held at about the level of the moth and sounded one or more times. In such a position it was absolutely impossible for the moth to see either the whistle or the experimenter. The

whistle was usually 180° from the moth; but occasionally it was placed ten to fifteen degrees away, but out of sight of the moth. In a few rare cases, for a special purpose, the whistle was blown in the presence of the moth. Whenever that was done it is indicated in the tables. The results of these experiments are recorded in Tables IV.–XI. The tables are self explanatory.

TABLE IV.

RESPONSES OF *Catocala flebilis* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	e^5	Flew.
2	1		Flew.
3	1		Flew.
4	1		Flew.
5	5		Flew.
6	3		Flew.
7	1		Flew.
8	1		Flew.
9	1		Flew.
10	1		Flew.
11	1		Moved its antenna, but did not fly.
12	1		Flew.

TABLE V.

RESPONSES OF *Catocala habilis* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	a^4	Flew.
2	1		Flew.
3	1		Flew.
4	1		Flew.
5	1		Flew.
6	1		Flew.

In the experiments recorded in this table (Table V.) the Galton whistle was held three feet from the tree on which the moth was resting.

TABLE VI.

RESPONSES OF *Catocala neogama* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	e^5	No response.
2	1		No response.
3	1	a^4	Flew (saw the whistle).
4	1	a^4	Flew.
5	1		Flew.

TABLE VII.

RESPONSES OF *Catocala piatrix* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	2	b^4	No response observed.
2	1		Flew.
3	1		Flew.
4	1		Moved its wings up and down
5	1		Ditto.
6	1		Flew.
7	1		No response observed.
8	1		Moved its wings up and down
9	1		Ditto.
10	1		Flew.
11	14		No response observed.
12	1		Moved its wing up and down.

TABLE VIII.

RESPONSES OF *Catocala relicta* var. *luctuosa* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	e^5	Made quivering movements with its wings.
2	1		Ditto.
3	1		Ditto.
4	1		Ditto.
5	1		Ditto.
6	1		Ditto.
7	1		Ditto.
8	1	a^4	Flew (It saw the whistle).
9	1		Whole body quivered.
10	1		Ditto
11	1		Ditto.
12	1		Ditto.
13	1		Ditto.
14	1		Ditto.
15	1		Ditto.
16	1		Ditto.
17	1		Ditto.
18	1		Ditto.
19	1		Ditto.
20	1		Ditto.
21	1		Ditto.
22	1		Ditto.
23	1		Moved antennæ gradually forward and then flew.
24	1		Body quivered.
25–35			Ditto.
36	1		Flew.
37	1		Whole body quivered.
38–43	1		Ditto.
44	1		Moved its antennæ four times and then flew.

TABLE IX.

RESPONSES OF *Catocala robinsoni* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1		Flew.
2	1		Flew.
3	1		Flew.
4	1		Flew.

TABLE X.

RESPONSES OF *Catocala vidua* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	a^4	No response observed.
2	1		Flew.
3	1		Flew.
4	1		Flew.
5	1		Flew.
6	1		No response observed.
7	1		Ditto.
8	1		Ditto.
9	1		Ditto.
10	1		Ditto.
11	1		Ditto.
12	1		Flew (It saw the whistle).
13	1		No response observed.
14	1		Flew.
15	1		Flew.
16	1		No response observed.
17	1		Flew.
18	1		No response observed.
19	1		Flew.
20	4		Flew.

TABLE XI.

RESPONSES OF *Catocala vidua*, SPECIMEN NUMBER 2, TO SOUND.

Number of the Experiment	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	a^4	Moved its antennæ.
2	1		Flew.
3	1		Flew.
4	19		Flew, but not until after the nineteenth whistle.
5	1		Flew.
6	4		No response noticed.
7	4		No response noted.
8	1		Flew (Saw the whistle).

In June, 1914, we made an attempt to see if, in the field, moths could be trained to respond to sounds to which they do not normally respond. We knew that this can be done in the laboratory. Our experience the year before had informed us that most Catocalæ do not respond to sounds of a low pitch. We selected an organ pipe giving 256 vibrations per second. This was sounded several times and if the moth did not respond it was sounded again and simultaneously one of us touched the moth with a brush. We then followed the moth to its next resting place and sounded it again, and if necessary, repeated it over and over.

For these experiments we used; *C. amica*, *C. epione*, *C. neogamma*, *C. ilia*, and *C. innubens*. With *innubens* and *epione* all results were negative. We found two specimens of *ilia* which responded to the sound of the pipe before they had been touched in any manner and one that did not so respond. This response from *ilia* was unexpected, but it militated against using it for these experiments. We succeeded in inducing one specimen of *amica* to respond to the pitch; but failed completely with two others. We experimented with five specimens of *C. neogama*, all males. We induced three individuals of *neogama* to respond to the sound of the organ pipe; but failed with two others. Although the cases in which we succeeded in inducing the moths to respond to sounds to which they do not usually react are few, the fact that we did succeed in a few cases supports our contention that these insects respond only to sounds that have a life significance.

Conclusions.

1. Our field experiments demonstrate that several different species of *Catocala* moths respond to certain high pitched notes of the Galton whistle; but that they usually do not respond to sounds of low pitch, such as the rumbling of trains, etc.

2. Most specimens responded to those high notes by flying to a nearby tree; but some, and this was especially true of *Catocala relicta*, responded by making quivering movements with its wings.

3. The degree of responsiveness was not the same in all species. Among the least responsive were *C. vidua* and *C. neogama;* at the other extreme were *C. flebilis*, *C. habilis*, and *C. robinsoni*.

4. We do not consider the failure of these moths to respond to certain sounds of low pitch a proof that they do not hear such sounds; indeed, we are inclined to believe that these creatures respond only to such sounds as have a life significance. Three things render this last assumption probable: (1) The fact that *C. unijuga*, which at first did not respond to whistling, did so readily after once a blast of air had been allowed to strike her body simultaneously with the sounding of the whistle; (2) that most of the natural enemies of these moths produce high pitched sounds and trains, and brass bands and other producers of low pitched or coarse sounds do not directly affect the survival of these moths; and (3) by carefully conducted field experiments, we were able to induce three specimens of *C. neogama* to respond to sounds to which the species does not usually react.

References.

The following list is not intended to be a complete bibliography of the sense of hearing of insects. It contains only such articles as have been referred to in the body of this paper. An effort has been made, however, to include all papers treating of experiments upon the auditory powers of the Lepidoptera. If any such have been omitted, it is because they have escaped our notice.

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